Novel Male Hormonal Contraceptive Combinations: The Hormonal and Spermatogenic Effects of Testosterone and Levonorgestrel Combined with a 5α-Reductase Inhibitor or Gonadotropin-Releasing Hormone Antagonist

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We postulated that the addition of a combined types I and II, 5α-reductase inhibitor (dutasteride) or long-acting GnRH antagonist (acyline) to combination testosterone plus levonorgestrel treatment may be advantageous in the suppression of spermatogenesis for male contraception. This study aimed to examine effects of novel combination contraceptive regimens on serum gonadotropins and androgens and sperm concentration.

This study was divided into three phases: screening (2 wk), treatment (8 wk), and recovery (4 wk). Twenty-two men (n = 5–6/group) received 8 wk of treatment with testosterone enanthate (TE, 100 mg im weekly) combined with one of the following: 1) levonorgestrel (LNG) 125 μg orally daily; 2) LNG 125 μg plus dutasteride 0.5 mg orally daily; 3) acyline 300 μg/kg sc every 2 wk (as a comparator for any additional progestin effects); or 4) LNG 125 μg orally daily plus acyline 300 μg/kg sc every 2 wk.

Serum gonadotropin levels were similarly suppressed by all treatments, falling to a nadir between 1.2 and 3.4% and 0.5 and 0.8% baseline for FSH and LH, respectively (*P* < 0.05). Serum dihydrotestosterone levels were significantly (*P* < 0.05) de-creased in the dutasteride group throughout the treatment period to a nadir of 31% baseline (wk 7). No significant differences in sperm concentrations among treatment groups were seen. Severe oligospermia (0.1–3 million/ml) or azoospermia was seen in none of five and four of five in TE + LNG; two of six and four of six in TE + LNG + dutasteride; two of six and four of six in TE + acyline; and one of five and three of five in TE + LNG + acyline groups, respectively. There was one non-responder in each of the TE + LNG and TE + LNG + acyline groups.

We conclude that the addition of a combined types I and II, 5α-reductase inhibitor or long-acting GnRH antagonist to a testosterone plus LNG regimen provides no additional sup-pression of gonadotropins or sperm concentration over an 8-wk treatment period. However, further evaluation of the effects of these regimens on the testis (including testicular steroid levels and germ cell maturation) and the treatment of larger numbers of men (and for longer periods) may provide data to support their place in contraceptive development. (*J Clin Endocrinol Metab* 90: 91–97, 2005)

A T PRESENT 580 MILLION couples worldwide employ family-planning methods, and it is expected that future demand for contraceptive options will continue to increase. The World Health Organization (WHO) has designated the development of new and improved methods of contraception for men and women as a key component in the strategy to improve the quality of family-planning programs (1). Currently the only approved methods of contraception available to men are condoms, which suffer from a relatively high failure rate (up to 12% in general use) (2), and vasal occlusion, which is not intended to be reversible.

Hormonal contraception provides the most promising

male-targeted option for the near future. Emerging data show that male hormonal regimens can provide contraceptive efficacy equal to that of the female oral contraceptive pill (3). The supporting evidence for this comparison now includes two WHO trials (4, 5) and more recently two efficacy studies using a testosterone-alone regimen (6) and a testosterone and progestin combination regimen (7) as safe and effective prototypes.

The rapid and maximal reduction in serum gonadotropin levels is a key consideration in designing effective male hormonal contraceptive (MHC) regimens. Progestins (P) appear to speed the rate of fall in serum gonadotropin levels when added to high-dose testosterone (T). However, they provide no greater depth of suppression with serum LH and FSH levels falling similarly to less than 0.3% (the limit of detection) and 1–2% baseline, respectively (8). It has also been demonstrated that when using more physiological T doses, the degree of gonadotropin suppression is enhanced by concomitant P administration (9). These data underlie the cur-

rent use of P in combined MHC regimens for its T dose-sparing effect, thereby eliminating excess androgenic side effects. Additionally, there is some evidence that P may also have nongonadotropin-mediated effects, which might contribute to suppression of spermatogenesis (10).

GnRH agonist and antagonist analogs have also been applied to MHC in an attempt to augment gonadotropin suppression. To date only GnRH antagonists have shown efficacy in suppressing sperm concentration (11–13). Recently we reported the first preliminary study in humans of a new GnRH antagonist, acyline [Ac-D2Nal-D4Cpa-D3Pal-Ser-4Aph (Ac)-Leu-Ilys-Pro-Dala-NH2] (14), which induced a rapid and sustained suppression of gonadotropins. It has been postulated that GnRH antagonists may be useful in the rapid induction of spermatogenic suppression (15). However, whether combinations of T with either P or GnRH antagonist produce equivalent outcomes or whether there is any benefit of the combination T plus P plus GnRH antagonist on the suppression of gonadotropins and spermatogenesis has yet to be directly tested.

The reduction of intratesticular androgen levels is also an essential element in the inhibition of spermatogenesis. LH suppression induced by MHC treatment profoundly suppresses intratesticular T levels (to ~2% control) but not that of its 5α-reduced metabolites, dihydrotestosterone (DHT) and androstanediol (16). This raises the possibility that DHT may contribute to androgen action within the testis and thereby support some degree of residual spermatogenesis. There are rodent data to support this concept and evidence that the type I 5α-reductase isoenzyme predominates in the testis (17, 18). However, previous human studies examining the effect of adding a type II 5α-reductase inhibitor (finasteride) to MHC regimens have not shown significantly greater suppression of sperm concentrations (19, 20). Recently, a dual types I and II 5α-reductase inhibitor (dutasteride) has become available for clinical use in the treatment of benign prostatic hypertrophy (21). Because this compound inhibits both isoenzymes of 5α-reductase, it may provide more complete withdrawal of DHT from the testis, resulting in greater impairment of spermatogenesis.

This study aimed to determine whether the addition of the dual types I and II 5α-reductase inhibitor dutasteride or that of the new GnRH antagonist acyline produced more rapid, extensive, and/or consistent suppression of gonadotropins and spermatogenesis when added to a conventional T plus P MHC regimen. To this end we performed a prospective, randomized, four-arm, 8-wk study of novel MHC combinations to identify potential promising strategies for clinical development.

Subjects and Methods

Subjects

Twenty-two men were recruited through media advertisement to participate in this study. The institutional review board of the University of Washington approved all study procedures, and subjects gave written informed consent before screening. The ethnicity of recruited subjects was 90% Caucasian. All men underwent medical interview, physical examination, and biochemical investigations. Subjects were required to fulfill each of the following criteria: 1) age 21–55 yr, 2) normal physical findings and normal testicular volumes, 3) two normal semen analyses according to WHO criteria (22), 4) normal serum FSH and LH, 5) normal serum T, and 6) normal liver and renal function and complete blood

count. Men with a past history of hypertension or significant cardiovascular, renal, hepatic, prostatic, and testicular disease or infertility were excluded. Men who were taking significant prescribed medications were also excluded as were men undertaking competitive sport who were subject to testing for androgen usage.

Study design

This research study was divided into three phases: a 2-wk screening phase, an 8-wk treatment phase, and a 4-wk recovery period.

After screening men were randomly assigned to one of the following four treatment groups (n = 5–6/group) for 8 wk: 1) testosterone enanthate (TE, Delstestryl, Bristol-Myers Squibb, Princeton, NJ) 100 mg im weekly + levonorgestrel (LNG, Wyeth, Madison, NJ) 125 μg orally daily, (n = 5); 2) TE 100 mg im weekly + LNG 125 μg orally daily + dutasteride (GlaxoSmithKline, Research Triangle Park, NC) 0.5 mg orally daily, (n = 6); 3) TE 100 mg im weekly + acyline (Multiple Peptide Systems, San Diego, CA) 300 μg/kg sc every 2 wk, (n = 6); and 4) TE 100 mg im weekly + LNG 125 μg orally daily + acyline 300 μg/kg sc every 2 wk, (n = 5).

In the treatment phase, serum FSH, LH, T, DHT, and estradiol (E2) levels were measured weekly before hormone injection administered by the clinical research staff. Semen analyses were also performed at each weekly visit after abstinence from ejaculation of at least 2 d. Men were asked to keep a medication log throughout the treatment phase at the end of which they underwent a previously planned vasectomy and testicular biopsy, the results of which are to be reported at a later date.

Acyline was originally synthesized by Jean Rivier (The Salk Institute, La Jolla, CA) (23). It is presently manufactured by Multiple Peptide Systems and distributed by the National Institute of Child Health and Human Development. In this study, the reconstitution of acyline for injection was undertaken by the University of Washington Pharmacy Department, no longer than 1 h before its administration. First, the lyophilized acyline product was loosened in the vial by gentle tapping and then slowly reconstituted by aseptically adding 2.2 ml of bacteriostatic water for a final concentration of 2 mg/ml. To minimize the formation of foam, shaking the vial during reconstitution was avoided. The vial was then carefully inspected for any evidence of particulate matter or gelling. During the course of this trial, no vial was rejected due to the acyline not being fully suspended. The number of injections administered at each 2-wk visit was based on the total volume of drug. Men received between three and four injections, depending on their body weight with no more than 4 ml injected at any one site.

Food and Drug Administration approval for acyline administration allowed for four consecutive doses, thus limiting the trial to 8 wk duration. Safety monitoring conducted throughout the study included a weekly clinical review, monthly serum biochemistry panel, and complete blood count. Men receiving acyline had additional surveillance with 2-wk liver function tests and 1 h of observation after their sc acyline injections for any adverse local or systemic effects.

Assays

Serum FSH and LH were measured by Delfia human (h)FSH kit (A0710201) and Delfia hLH Spec kit (A031–101; Perkin-Elmer, Wallac, Inc., Gaithersburg, MD). The sensitivity of the FSH assay was 0.016 IU/liter with an interassay variation for low, mid, and high pools of 16.8, 5.6, and 4.3%, and intraassay variation of 8.3, 3.4, and 2.3%. The mean concentration for each pool was 0.054, 0.94, and 15.1 IU/liter. The sensitivity of the LH assay was 0.019 IU/liter with an interassay variation for low, mid, and high pools of 20.5, 8.3, and 9.6% and intraassay variation of 10.5, 6.2, and 5.0%. The mean concentration for each pool was 0.055, 1.05, and 20.6 IU/liter.

Sensitive LH was also determined by our sensitive immunofluorometric assays as previously published (8). These procedures use modifications of the commercial LH (Delfia, Wallac, Turku, Finland) immunofluorometric assay resulting in increased sensitivity (0.01 IU/liter) with an interassay variation of 5%.

T was measured by total testosterone coated tube ^{125}I RIA kit (TKTT5; Diagnostic Products Corp., Los Angeles, CA) with a sensitivity of 0.35 nmol/liter and interassay variations for low, mid, and high pools of 13.6, 6.1, and 6.8% and intraassay variation of 10.0, 5.3, and 6.6%. The mean concentration for each pool was 3.9, 10.8, and 24.8 nmol/liter.

SHBG was measured by Delfia hSHBG kit (A070–101, Perkin-Elmer,

Wallac) with a sensitivity of 0.2 nmol/liter and an interassay variation for low, mid, and high pools of 31, 10.6, and 6.8% and intraassay variation of 3.8, 1.7, and 2.2%. The mean concentration for each pool was 7.0, 21.6, and 117 nmol/liter.

Estradiol was measured by third-generation estradiol double antibody 17β-estradiol ^{125}I RIA kit (DSL-39100; Diagnostic Systems Laboratory, Webster, TX) with a sensitivity of 5.5 pmol/liter and interassay variations for control 1 and control 2 pools of 7.0 and 8.9% and intraassay variation of 5.6 and 5.3%. The mean concentration for control 1 pool was 41 and for control 2, 115 pmol/liter.

DHT was measured by active dihydrotestosterone ^{125}I RIA kit (DSL-9600, Diagnostic Systems Laboratory) with a sensitivity of 0.043 nmol/liter and interassay variations for control 1 and control 2 pools of 25 and 19% and an intraassay variation of 9.9 and 11%. The mean concentration for control 1 pool was 0.23 and for control 2, 1.7 nmol/liter.

Statistical analyses

Data are shown as mean ± sem. Descriptive statistics are presented via the median and corresponding 95% confidence intervals. Statistical comparisons were made using SPSS (SPSS Inc., Chicago, IL). Undetectable assays were assigned the value of assay sensitivity. Because data appeared nongaussian, nonparametric techniques have been applied. Data were transformed to the ratio of observation at time t to baseline. Comparison of these ratios within and among the four treatment groups was made via the Kruskal-Wallis test. Significant results were further examined through Bonferonni-type comparisons.

Results

Baseline characteristics

As outlined in Table 1, no differences in mean age, testicular volume, sperm concentration or motility, ejaculate volume, or serum reproductive hormones among treatment groups could be determined. Likewise, mean body mass index was between 25 and 27 and similar in all groups.

Serum gonadotropin levels

Serum gonadotropin levels declined significantly over the 8-wk treatment period in all groups, falling to nadir of between 0.03 and 0.14 IU/liter and 0.019 and 0.03IU/liter for FSH (Fig. 1A) and LH (Fig. 1B), respectively. No differences in gonadotropin levels among groups were detected. Because many of the serum LH values fell to the limit of assay detection, a 2-fold more sensitive assay method was applied to explore whether differences among treatment groups could be detected; however, none were found.

Semen analysis

Sperm concentration fell significantly from baseline in all but the TE + LNG + acyline group over the 8-wk treatment period (Fig. 2). The lack of significant decrease observed in this group (n = 5) is attributable to a nonresponding subject whose sperm concentration ranged between 12.3 and 60 mil-
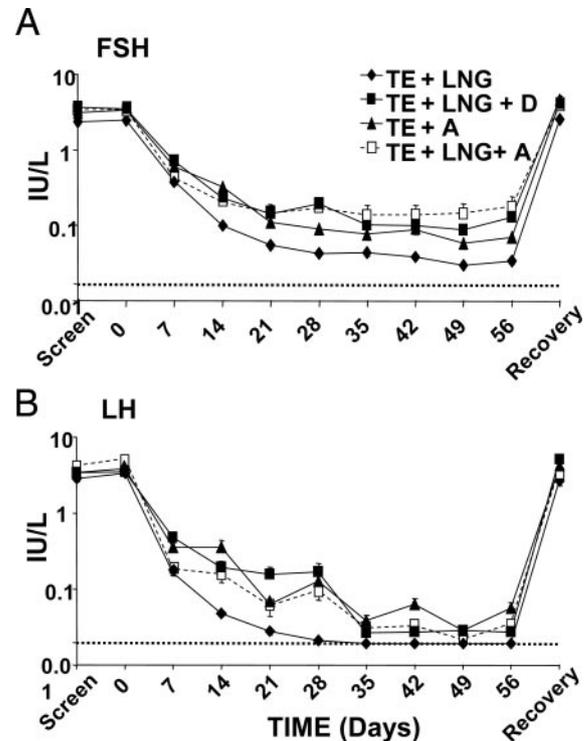

FIG. 1. Changes in serum gonadotropins over the 8-wk treatment phase in 22 men (n = 5–6/group) randomized to receive TE (100 mg im weekly) combined with: 1) LNG 125 μg orally daily; 2) LNG 125 μg plus dutasteride (D) 0.5 mg orally daily; 3) acyline (A) 300 μg/kg sc every 2 wk; or 4) LNG 125 μg plus A 300 μg/kg every 2 wk. Serum FSH (A) and LH (B) are shown at screening and then weekly thereafter. Serum FSH and LH decreased significantly d 7–56 in all groups (*P* < 0.05). No differences in gonadotropins were detectable among treatment groups at any single time point. Data are graphed logarithmically and shown as mean ± sem, n = 5–6/group. *Dashed line* indicates threshold of assay sensitivity, 0.016 and 0.019 IU/liter for FSH and LH, respectively.

lion/ml throughout the treatment phase of the study. All treatment groups reached a mean sperm concentration of less than 3 million/ml between wk 6 and 8, but only the TE + LNG + dutasteride and TE + acyline treatment groups achieved a mean sperm concentration of less than 1 million/ml during the study. On the day of vasectomy (d 56 of treatment), the number of men reaching severe oligospermia (0.1–3 million/ml) and azoospermia, respectively, in each group was TE + LNG (none of five and four of five), TE + LNG + dutasteride (two of six and four of six), TE + acyline (two of six and four of six), and TE + LNG + acyline (one of five and three of five). The number of nonresponders (>3 million/ml) in each group was TE + LNG (one of five), TE

TABLE 1. Baseline characteristics of the subjects at enrollment

Group	Age (yr)	Combined testis volume (ml)	Sperm conc (10^6/ml)	Serum FSH (IU/liter)	Serum LH (IU/liter)	Serum T (nmol/liter)	Serum SHBG (nmol/liter)	Serum E2 (pmol/liter)	Serum DHT (nmol/liter)
TE + LNG (n = 5)	36.0 ± 0.8	59 ± 1	98 ± 14	2.3 ± 0.1	2.8 ± 0.3	14 ± 0.7	22.4 ± 1.9	208 ± 7.0	1.8 ± 0.3
TE + LNG + D (n = 6)	34.3 ± 0.9	50 ± 1	81 ± 8	3.7 ± 0.3	3.4 ± 0.2	14 ± 0.8	23.3 ± 1.2	202 ± 5.9	1.1 ± 0.03
TE + A (n = 6)	36.2 ± 1.7	52 ± 1	78 ± 9	3.1 ± 0.3	3.4 ± 0.2	14 ± 1.0	33.2 ± 3.5	202 ± 12.3	1.4 ± 0.03
TE + LNG + A (n = 5)	37.2 ± 1.3	55 ± 2	49 ± 4	3.4 ± 0.4	4.2 ± 0.3	15 ± 1.2	43.6 ± 5.5	192 ± 7.2	1.2 ± 0.1

Results are shown as mean ± sem. All treatment groups are n = 5–6. No differences were seen between groups for any baseline parameter, *P* < 0.05. D, Dutasteride; A, acyline; Conc, concentration.
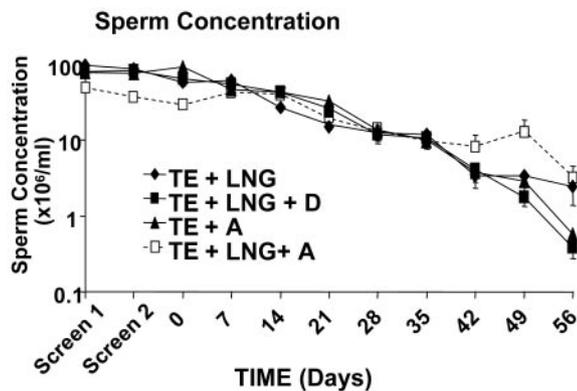
Sperm Concentration

FIG. 2. Changes in sperm concentration after 8-wk treatment phase with TE (100 mg im weekly) combined with: 1) LNG 125 μg orally daily; 2) LNG 125 μg plus dutasteride (D) 0.5 mg orally daily; 3) acyline (A) 300 μg/kg sc every 2 wk; or 4) LNG 125 μg plus A 300 μg/kg every 2 wk for 8 wk. No significant differences among groups over the treatment period were observed. Data graphed logarithmically and shown as mean ± SEM, n = 5–6/group.

+ LNG + dutasteride (none of six), TE + acyline (none of six), and TE + LNG + acyline (one of five). In all cases, when a single sperm concentration less than 3 million was achieved, there was no rebound above this threshold. Thus, one could define responders based on a single value using this threshold. With regard to azoospermia (<0.1 million/ml), a slight increase was seen in only one man with a rebound to 3 million/ml followed by concentrations of 0.1–0.2 million/ml on three subsequent samples.

Ejaculate volume was unchanged in all subjects, and thus neither this parameter nor the derivative, total sperm count was used in the analysis. Sperm motility was unable to be assessed after a median of 35 d from the institution of therapy. It is noted that up until 28 d, the median motility was not obviously different from baseline.

Baseline characteristics for the nonresponder (both Caucasian) in the TE +LNG group were within 1 SD of the group mean for all parameters except age. For this characteristic the subject was outside 1 SD at 43 yr, compared with a group mean of 36 ± 4 yr. The nonresponder in the TE + LNG + acyline group also displayed baseline characteristics outside the group mean ± 1 SD with an FSH of 5.9 (group mean 3.4 ± 1.8) and DHT of 2.1 (group mean 1.2 ± 0.7).

T and SHBG

Serum T levels remained in the normal range (7.6–22 nmol/liter) throughout the treatment phase of the study except in the TE + acyline treatment group at d 7 when the mean T level fell significantly ($P < 0.05$) to 6.4 nmol/liter (Fig. 3A, NS, compared with other groups). No other differences in T levels were seen within or among treatment groups over the treatment phase of the study. Serum SHBG levels also remained in the normal range (3.2–47 nmol/liter) but declined significantly ($P < 0.05$) in all groups as follows: TE + LNG (d 7–21, 35–56), TE + LNG + dutasteride (d 14–56), TE + acyline (d 28, 56), and TE + LNG + acyline (d 7–56). Significantly higher SHBG levels were seen in the TE + acyline group from all other groups on d 35 and 42 ($P =$

0.03–0.04) and all but the TE + LNG group on d 56 ($P = 0.03$), with no other differences among groups observed.

DHT

Serum DHT levels fell significantly from baseline in the TE + LNG + dutasteride treatment group (0.38–0.45 nmol/liter, d 7–56, $P < 0.05$, nadir 31 ± 3% baseline) and the TE + acyline group (0.68 nmol/liter, d 7, $P < 0.05$, 68 ± 6% baseline). Significant differences among groups were observed, with DHT levels in the TE + LNG + dutasteride group being significantly lower than all other groups, d 14–56 ($P < 0.01$–0.04).

E2

Serum E2 levels remained in the normal range (76–353 pmol/liter) throughout the treatment phase of the study. E2 levels did not change significantly from baseline and no differences were seen among groups (Fig. 3D).

Recovery and adverse events

Recovery of gonadotropin and T levels was seen in all 22 men by 7 wk post vasectomy and biopsy. No serious adverse events occurred, and all treatments were well tolerated. Eight of 11 men receiving acyline experienced mild erythema, swelling, and itch at the injection site. In all men this reaction was limited and generally lasted less than 24 but no longer than 48 h. This reaction was not associated with systemic features and tended to improve with each subsequent 2-wk dosing. One man in the TE + acyline group experienced a transient elevation in liver function tests (2–3 times the upper limit of normal) for 2 months from his last injection. Subsequent testing for intercurrent viral illnesses including cytomegalovirus, Epstein-Barr virus, and hepatitis A, B, and C was negative. At present the etiology of this observed abnormality remains unclear. Four men complained of fluctuations in mood (two in each of the TE + LNG + dutasteride and TE + LNG + acyline groups), but in all cases this was mild and resolved spontaneously during either the treatment or recovery phases.

Discussion

In this study we examined two novel MHC strategies aimed at improving the speed and extent of suppression of serum gonadotropin levels and sperm concentration. We have shown that neither the addition of a dual types I and II 5α-reductase inhibitor, dutasteride, or that of the new potent GnRH antagonist, acyline, to an established MHC regimen of TE + LNG (24) provided benefits in terms of these primary end points over the 8-wk period of study. We also report for the first time the effects of repetitive sc administration of acyline by injection and have shown that when given with TE, it produces similar, but not better, suppression of gonadotropins than that of the conventional TE + LNG regimen.

All treatments were similarly effective in terms of both the rapidity and depth of gonadotropin suppression. FSH levels remained detectable in all groups at 1.2–3.4% baseline. LH levels were also suppressed by all treatments to below or
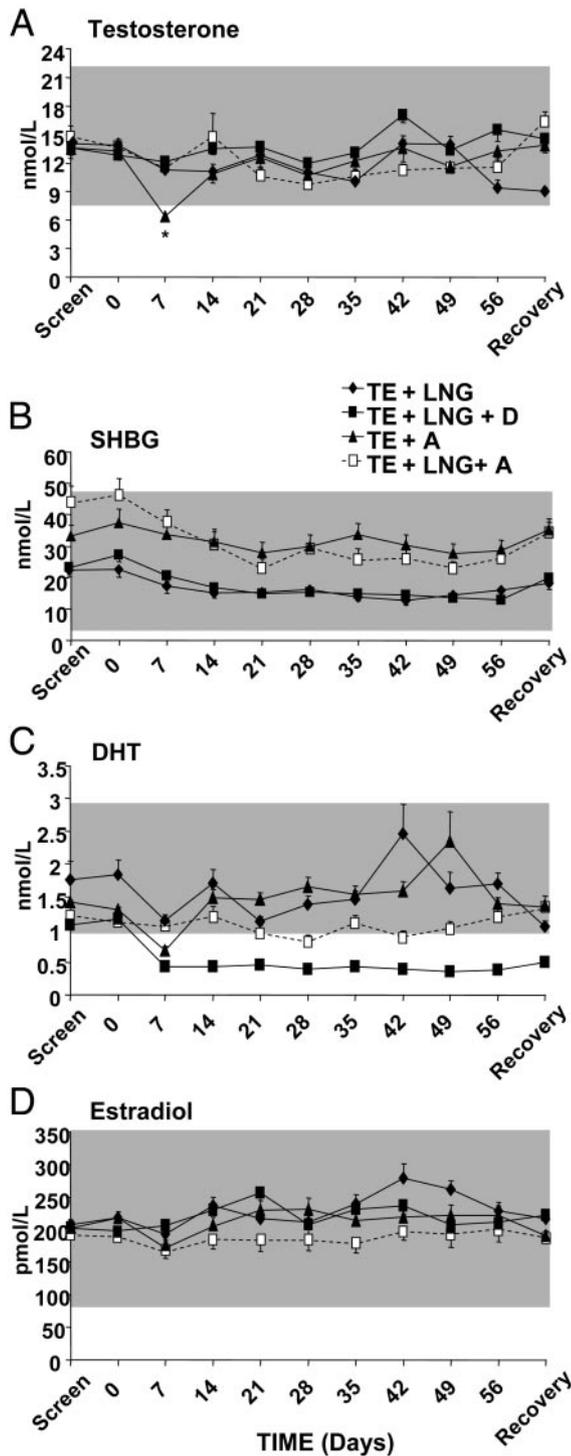
FIG. 3. Changes in serum T (A), SHBG (B), DHT (C), and E2 (D) levels in 22 men (n = 5–6/group) randomized to receive TE (100 mg im weekly) combined with: 1) LNG 125 μg orally daily; 2) LNG 125 μg plus dutasteride (D) 0.5 mg orally daily; 3) acyline (A) 300 μg/kg sc every 2 wk; or 4) LNG 125 μg plus A 300 μg/kg every 2 wk for 8 wk. Serum levels shown at screening and then weekly thereafter. Data are shown as mean ± SEM, n = 5–6/group, with *shaded area* delineating normal ranges. A, T levels did not change significantly from baseline and remained in the normal range throughout the study period except in the TE + A group at d 7 (6.4 nmol/liter, *, $P < 0.05$). No differences in T levels among groups were detected at any time point. B, SHBG levels remained in the normal range (3.2–47 nmol/liter) throughout

near the limit of assay detection, including that of the supersensitive modified Delfia system (8). The extent of gonadotropin suppression was comparable with that previously reported with the use of other T/P combinations (16, 25–27) but was better than that seen using the GnRH antagonist, Nal-Glu combined with either low- or high-dose TE (11, 28, 29). Previous work (14) demonstrated greater potency and longer duration of action of acyline, compared with the experimentally applied antagonists, Nal-Glu and Nal-Lys, and those available commercially, cetrorelix and teverolix, in addition to it having comparatively few side effects. As previously shown, the TE + LNG protocol (24) produced a rapid and profound reduction in serum LH and FSH. Thus, in this context it is perhaps not surprising that the addition of acyline failed to show further gonadotropic suppression. At this time there seems no advantage to considering the three agent combination of TE + LNG + acyline as offering any advantage over the TE + LNG combination.

It is clear that FSH remains detectable in serum despite treatment with sex steroids and/or GnRH antagonists, suggesting that a small but potentially significant component of pituitary FSH secretion is GnRH independent. On the other hand, to the extent possible using extremely sensitive gonadotropin assays, LH is abolished from serum, indicating that it is entirely GnRH dependent (8). However, what remains uncertain is the possibility of LH-independent testicular steroidogenesis and the role it may play in maintenance of constitutive spermatogenesis similar to that seen in the LH receptor knockout mouse model (30).

As previously reported (14), acyline induced skin changes at the site of injection that included erythema and, in some cases, mild induration. These skin reactions tended to improve with each subsequent 2-wk dose such that by the fourth set of injections, little if any reaction was seen. Serum gonadotropins and T levels returned to baseline levels in all patients receiving acyline by 7 wk after their last treatment visit.

The combination of TE + acyline was as effective as TE + LNG but failed to show any advantage in terms of rapidity of gonadotropin suppression and induction of oligo/azoospermia over the 8-wk period. It has been postulated that GnRH antagonists may provide better induction of contraception, compared with P (15), but this was not evident in the present study. Acyline may offer other advantages in terms of compliance, dosing interval, differing side effect profile, and possible quicker offset of action (7). However, there remain significant problems in terms of its reconstitution for injection and likely higher cost, compared with progestagenic agents.

the treatment phase of the study but declined significantly ($P < 0.05$) in all groups as follows: TE + LNG (d 7–21, 35–56), TE + LNG + D (d 14–56), TE + A (d 28, 56), and TE + LNG + A (d 7–56). SHBG levels in the TE + A group were significantly higher than the other treatment groups, d 35 and 42 ($P = 0.03$–0.04) and all but the TE + LNG group, d 56 ($P = 0.03$). C, DHT levels fell significantly ($P < 0.05$) from baseline in the TE + LNG + D (31–41% baseline, d 7–56) and TE + A groups (48% baseline, d 7). DHT levels were significantly lower in the TE + LNG + D group, d 14–56 ($P < 0.01$–0.04) than the other treatment groups. D, E2 levels remained in the normal range (76–353 pmol/liter) in all groups throughout the treatment phase of the study. No differences from baseline or between groups were observed.

Sperm concentrations were similarly suppressed by all treatments with the proportion of men falling below the thresholds of azoo- or severe oligospermia not varying among treatment groups. All men in the TE + LNG + dutasteride and TE + acyline groups suppressed their sperm concentration to less than 3 million/ml. In contrast, there were two nonresponders at the time of vasectomy (d 56), one in each of the TE + LNG and TE + LNG + acyline groups (sperm concentrations of 12 and 15 million/ml, respectively). Analysis of these individual nonresponders' clinical and endocrine response parameters showed one individual was older and the other had higher baseline FSH and DHT levels. These hormonal results are contradictory to previous larger studies examining for differences between azoospermic and oligospermic responders to T administration, which have shown higher pretreatment FSH levels in azoospermic responders (31, 32) and no differences in pretreatment DHT levels (33, 34). There remains an apparent lack of consistent identifiable differences in hypothalamopituitary response to MHC treatment in nonresponders, which perhaps reflects a need to examine testicular end points such as stereology, steroid levels, and patterns of gene expression to understand individual patterns of spermatogenic suppression. To date, only a rise in 5α-reductase activity during treatment (33, 34) and an increased prevalence of extended CAG repeats on the androgen receptor have been shown to be associated with nonresponse within the normal fertile population (35).

In terms of spermatogenesis, MHC treatment results predominantly in early spermiation failure followed by a decrease in the spermatogonial number available to undergo differentiation (16, 36). One can speculate that the initial fall in sperm concentration (within the first month) may result from spermiation failure followed by a progressive and profound secondary fall due to reduced progression of germ cell maturation. This raises the possibility that germ cell populations in the different groups may be different, even though the sperm output over the short 8-wk period of study showed no differences.

In normal men approximately 5% of T is 5α reduced to DHT (37, 38), 0.6% is aromatized to E2 (39, 40), and the remainder is cleared directly through the liver. The use of either finasteride or dutasteride in normal elderly men results in a decrease of serum DHT to between 5 and 15% of baseline (21, 41–43) with a concomitant rise in T to between 104 and 121% baseline (21, 41, 43). In one study of finasteride 5 mg daily, it was suggested that this rise in T may be due to decreased clearance through the inhibited 5α-reductase pathway rather than to an increase in LH (41). A lack of significant LH increase in association with a 21% increase in serum T has also been more recently confirmed using dutasteride 0.5 mg over a 6-month period (43), supporting the notion that T clearance is reduced with the use of 5α-reductase inhibitors. In our current study, men had clamped LH secretion as a result of sex steroid and acyline treatment and were administered a fixed amount of exogenous T (100 mg TE weekly). Serum DHT fell to only 31% baseline after 7 wk of treatment in the TE + LNG + dutasteride group, a more modest decrease than that seen in normal older men. We speculate that this may be due to increased substrate avail-

ability as a result of increased serum total and free T in the week after im TE (44, 45).

It is reasonable to assume (but not directly proven) that dutasteride will impair DHT production within the testis. It could be proposed that the small increase in serum T seen in normal men may also result in a similar rise in intratesticular T with dutasteride due to reduced T clearance within the testis, which in turn may maintain a level of spermatogenesis. But in our LH-clamped MHC model, this concept was not support by the data showing that sperm concentrations are increased.

Whereas no differences in gonadotropins or sperm concentrations were apparent with the use of these two novel agents, they are worthy of further consideration for several reasons. First, this trial was of short duration (less than one cycle of the seminiferous epithelium) and the number of subjects small. Thus, we may have missed demonstrable long-term changes of relevance to contraceptive development. In particular, given more time, further falls in sperm concentration may have been evident. Second, acyline was effective at 2-wk dosing intervals, making it potentially attractive as an agent for induction of spermatogenic suppression and in terms of patient compliance, particularly when combined with a long-acting T preparation. Third, dutasteride significantly reduced serum DHT levels and therefore may be of potential benefit in reducing the effect of exogenous T administration on such organs as the prostate. Finally, a closer examination of the intratesticular environment using quantitative stereology and steroid hormone level measurement may yet reveal differences and associations not apparent using serum hormone and seminal parameters.

Acknowledgments

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